The Effect of Low Temperature on Flowering of *Rhodanthe Floribunda*

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ABSTRACT— ***Rhodanthe floribunda is a potential Australian native flower species. Studying the influences of chilling duration and seedling ages at chilling on flowering is important to develop a novel species for commercialization. The study was conducted in southern Queensland, Australia during 28 September 2009 to 1 March 2010. Seedlings of four age groups (1, 7, 14, 28 days old) were exposed to different cooling periods (0, 3, 7, 14 and 21 days) at 20/10^{0}C under short day (11h). It was found that R. floribunda has a facultative requirement for flowering in response to low temperature. The species could perceive vernalization at a very early stage, suggesting a short juvenile phase of this species. The longest chilling duration and the oldest seedlings prior to chilling had faster development rate and were more floriferous.***

Keywords— Rhodanthe floribunda, Chilling, Age, Visible bud stage, Anthesis, Australian species.

1. INTRODUCTION

Temperature is reported to influence floral initiation and subsequent development of the species of Asteraceae family (Bunker 1995). In which, some require a certain period of low temperature (vernalization) to induce synchronous flowering (Damann & Lyons 1996; Zoberi et al. 2003), and flowering can take place in both LDs and SDs after chilling treatment (Bender et al. 2002; Bunker 1995).

Rhodanthe floribunda (DC) Wilson (syn. *Helipterum floribundum*) is a species of Asteraceae family. *R. floribunda* is commonly called the white sunray or white paper daisy that is found in semi-arid areas of Queensland, New South Wales, South Australia, Northern Territory and Western Australia (Barker et al. 2002). It is a floriferous and attractive plant with potential as potted colour species for local and export market (Johnston & Joyce 2009). Bunker (1995) reported that *R. floribunda* was a facultative long day (LD) plant. Other previous research conducted on *R. floribunda* includes physiological seed dormancy (Hoyle et al. 2008), effects of light, temperature and gibberellic acid (GA) on germination (Plummer & Bell 1995; Plummer et al. 1997), and the effects of day-length and light supplementation on flowering (Bunker 1995; Roberts et al. 2005). Roberts (2005) showed that low minimum temperatures (below 10˚C) experienced in April, May and June plantings in south east Queensland were found to reduce the time to the first visible bud initiation.

The aim of this study was to quantify the amount of chilling required and to determine whether the seedling ages at chilling influenced flowering.

2. MATERIALS AND METHODS

2.1. Plant materials and growing media

All seeds used were collected at Wallen Station in south western Queensland (GPS: 27˚57'748"S; 148˚ 00'834"E) on 14th September 2003. Seeds were cleaned and stored in the cold room of Queensland Seed Technology Laboratory at 5°C until required.

Seeds were sterilized with 2 g L^{-1} chlorine sown into 9-cm diameter plastic Petri dishes containing 10g L^{-1} Agar with 50 mg L^{-1} GA_3. Petri dishes were sealed with parafilm to avoid seed desiccation prior to placement in an air conditioned room at 25^{0}C until germination. Seeds were planted sequentially to provide seedlings of the appropriate ages for the experiment.

Germinated seeds were then planted into 100-cell trays containing propagation medium of peat (TM Marketing Pty Ltd., Torrens Park, SA, Australia), perlite (Chillagoe Perlite, Mareeba, QLD, Australia) and vermiculite (Peter Bacon Enterprises, Rocklea, QLD, Australia) with 2 g L^{-1} Basacote® Mini 3 month [N:P:K = 13:6:16] (Compo do Brazil S.A, Brazil) in greenhouse bays according to scheduled treatments.

Seedlings were held in a short day (SD) bay at 30/20^{0}C before being exposed to different cooling periods. After 2 weeks, seedlings were transplanted individually to 100mm (0.5 L) diameter plastic pots containing growth media of 100% composted pine bark (Basset Barks Pty Ltd., Glasshouse Mountains, QLD, Australia) with 2 g L^{-1} Osmocote® plus 8-9 month (NPK: 15-3.9-9.1 plus 1.5Mg and TE), 1 g L^{-1} Osmocote® plus 3-4 month [N:P:K 16:5:9.2 + 1.8 Mg and TE], 2 g L^{-1} Nutricote® [N:P:K 16:4.4:8.3] (Chisso-Asahi Fertilizer Co.,Ltd. Tokyo, Japan), 1.3g L^{-1} Osmoform® [N:P:K 18:2.2:11 + 1.2Mg] (Scotts Australia, Baulkham Hills, NSW, Australia), 1.3 g L^{-1} Coated iron [Fe:S 28:17], 1.2 g L^{-1} Dolomite ® [Ca:Mg 14:8] (Yates, Australia) and 1.2 g L^{-1} Saturaid ® (Debco, Melbourne, Australia).

2.2. Treatments, Data Collection and Analysis

Two bays in the research greenhouse at University of Queensland Gatton nursery were used and were set at a temperature of 20/10 and 30/20^{0}C (day/night) under short days of 11 hours of sunlight from 6:00am – 5:00pm; at this time the blackout curtain in each bay was closed. Humidity and temperature sensors (Vaisala®, Finland) were used to record the temperature and humidity in each bay every 15 minutes. The light intensity of the greenhouse bay was 380 ± 44 µmol m^{-2} s^{-1}. The experiment started from 28 September 2009 and terminated on 1 March 2010.

Four age groups of seedlings (1, 7, 14 and 28 days old) were exposed to different cooling periods at 20/10^{0}C under SDs: 0 (without cooling), 3, 7, 14 and 21 days prior to transfer to 30/20^{0}C with 10 single replicate plants allocated for each treatment. Plants were observed every two days and the number of days to first visible floral bud (FVFB) and anthesis, and the number of branches at FVFB was recorded. The number of inflorescences per plant was recorded at week 6, 12 and 23. A completely randomized design was used. Data obtained were subjected to analysis of variance using the General Linear Model procedure in Minitab® version 15.

3. RESULTS

Seedlings that were chilled for 7 to 21 days reached the visible bud (VB) stage in 42 - 47 days, significantly earlier ($P< 0.05$) than the control (54 days) and those that were chilled for 3 days (62 days) (Table 3.1). Chilling for 21 days significantly reduced ($P < 0.05$) the time to anthesis (60 days) compared to the control (67 days) and chilling for 3 days which greatly delayed time to anthesis (81 days) (Table 3.1).

Plants that received the chilling treatment as 1-day old seedlings reached the VB stage in 55 days, significantly more ($P < 0.05$) than 1 week old seedlings (47 days) but similar to 2 and 4 week old seedlings. However, time to anthesis of 4 weeks old (62 days), 2-week old (67 days) and 1-week old seedlings (65 days) were significantly shorter ($P < 0.05$) than that of 1-day old seedlings (74) (Table 3.1).

In addition, 5% plants chilled for 0, 3 and 7 days did not reach the VB stage by the end of experiment (23 weeks from planting); and 12.5%, 17.5% and 2.5% plants initiated flower buds but did not reach anthesis, respectively.

Table 3.1. Effects of plant ages and chilling duration on floral development of *R. floribunda*

Treatment	Days to first visible bud (VB)	Days to anthesis	Inflorescences per plant at week 6	Inflorescences per plant at week 12	Inflorescences per plant at week 23	Inflorescences dried weight per plant (gram)
Chilling duration						
0 day	54.52 (a)	67.13 (b)	0.6 (ab)	18.9 (ab)	31.82 (a)	0.115 (a)
3 days	61.73 (b)	80.84 (c)	0.2 (a)	13.8 (a)	34.40 (a)	0.106 (a)
7 days	47.01 (c)	62.85 (ab)	0.7 (ab)	29.1 (ab)	38.39 (a)	0.130 (a)
14 days	47.48 (c)	64.78 (ab)	0.8 (ab)	32.8 (b)	50.16 (ab)	0.159 (ab)
21 days	42.60 (c)	59.63 (a)	1.3 (b)	52.3 (c)	68.52 (b)	0.224 (b)
P-value	*	*	*	***	*	*
Age						
1 day	55.23 (a)	74.11 (c)	0.7 (a)	14.9 (a)	18.33 (a)	0.041 (a)
1 week	47.42 (b)	64.92 (ab)	0.7 (a)	24.1 (ab)	24.44 (a)	0.060 (ab)
2 weeks	49.69 (ab)	67.32 (b)	0.5 (a)	30.9 (b)	52.48 (b)	0.146 (b)
4 weeks	50.34 (ab)	61.83 (a)	0.8 (a)	47.5 (c)	83.38 (c)	0.340 (c)
P-value	*	*	n.s.	***	***	***
Chilling*Age	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

*Experiment was terminated after 23 weeks from planting. Flowers were dried at 60^0C for 24h. Values followed by different letters within a column are significantly different according to Tukey test and simple t-test. n.s.: not significant, * P<0.05, **P<0.01, ***P<0.001.*

Plants chilled for 3 days had an average of 0.2 inflorescences per plant, significantly lower (P < 0.05) than that of plants chilled for 21 days (1.3) but similar to other treatments at week 6. At week 12, plants that received 21 day chilling had the highest number of inflorescences/plant (52.3), significantly greater (P < 0.01) than the14-day cold treated plants (32.8 inflorescences/plant) which was significantly higher than 3-day chilled plants (13.8) which was similar to the control (18.9) and plants receiving 7-day chilling. However at week 23 when the experiment ended, non-chilled plants, 3 and 7 day cold induced plants had similar number with 32, 34 and 38 inflorescences/plant, respectively; while plants that were chilled for 21 days had the higher number (P < 0.05) (69 inflorescences/plant) which was higher but not significantly higher than and 14 day chilled plants which had 50 inflorescences/plant (Table 3.1).

When averaged over chilling duration, plants from each age group had a similar number of inflorescences at week 6 after planting with 0.5 - 0.8 inflorescence/plant. At week 12, plants that were chilled as 4-week old seedlings had the highest number (P < 0.001) of inflorescences/plant (46), while those chilled as 1-day seedlings had 15 inflorescences/plant had significantly fewer than those chilled as 2-week old seedlings (31) but similar to those chilled as 1 week old seedlings (24). Similar results were obtained at the end of the experiment (23 weeks) with plants chilled as 4-week old seedlings having 83 inflorescences/plant followed by plants chilled as 2-week old seedlings (53), while there were no significant difference between plants chilled as 1-day old seedlings (18) and 1-week old seedling (24) (Table 3.1).

As expected, inflorescence dried weight of plants that were chilled for 21 days (0.224g) was significantly higher (P < 0.05) than those chilled for 0, 3 and 7 days with 0.115, 0.106 and 0.130g/plant respectively, but similar to the plants chilled for 14 days (1.159g/plant) (Table 3.1). Moreover, chilling was more effective for older plants. Plants that were chilled as 4-week old seedlings showed the highest inflorescence dry weight (0.340g) (P < 0.001), followed by 2-week old seedlings (0.146g) which was higher than 1-day old seedlings (0.041g), but similar to that of 1-week old seedling (0.060g) (Table 3.1).

Also, there were no interaction between chilling duration and plant age in relation to floral development parameters of *R. floribunda* (Table 3.1).

4. DISCUSSIONS

4.1. Effects of chilling duration on flowering

Plants of *R. floribunda* flowered without chilling and hence it has a facultative requirement for low temperature (Finnegan et al. 1998; Michaels & Amasino 2000; McDonald & Kwong 2005).

Five percent of plants remained vegetative in the non-chilled control and those plants chilled for 3 and 7 days, and 12.5%, 17.5% and 2.5% plants, respectively, did not reach anthesis; while all plants that received 14 and 21 day chilling flowered, reaching first VB in shorter time than control and plants chilled for 3 days. These results are in agreement with Gleichsner & Appleby (1996) who found that longer chilling duration (to a limit) reduces the time to flowering of ripgut brome (*Bromus diandrus*). Similarly, Pearson *et al.* (1995) also found that at least 2-week duration of cold at 12^0C accelerated floral development of Cape daisy. The results presented in this study further confirmed the role of low temperature in promoting early flowering of *R. floribunda* as reported by Roberts *et al.* (2005).

Chilling for 3 days resulted in the longest time to first VB and anthesis (Table 3.1), suggesting that a short duration of chilling might not be enough to induce a stable floral induction stage as is reported in many vernalization studies (Michaels & Amasino 2000; McDonald & Kwong 2005; Taiz & Zaiger 2006) where the common temperature range of 0 - 7^0C were used for vernalization, while chilling temperature ($20/10^0$C) used in this study was not in the range reported.

McDonald & Kwong (2005, p. 98) stated that plants can be devernalized under hot temperature following a short period of vernalization (usually less than five days). Further, the devernalizing effect of hot temperature decreases in accordance with the increase of vernalization duration (Michaels & Amasino 2000; Taiz & Zaiger 2006), thus plants might not be devernalized if they have achieved a saturated and stable state. Some authors suggested that devernalization can be prevented by placing plants that has just been vernalized into a 'neutral' temperature (around 15^0C) for several days (Yeh et al. 1997; Hopkins & Huner 2009; Cave & Johnston 2010). Furthermore, Sun et al. (2008) showed that reduction of photosynthesis and stomatal conductance of chrysanthemum resulted from sudden change of temperature from $23/18^0$C to $33/28^0$C (D/N). In this study, plants that were transferred to $30/20^0$C after 3-day chilling were younger than other plant groups under 7, 14 and 21 days, thus, they might have been more susceptible to this abrupt change of temperature.

In addition to the effect on flower development, chilling influenced total inflorescence number and weight. Plants that

received chilling as 21-day old seedlings had more inflorescences and a higher inflorescence weight than the control plants and plants chilled for 3 and 7 days (Table 3.1). This result is consistent with the results reported by several authors who concluded that a certain period of low temperature is needed to promote flower development; shorter durations do not influence flowering (Pearson et al. 1995; Horva´th et al. 2003) or flowering is less effective (King et al. 1992; Michaels & Amasino 2000; Samach & Coupland 2000).

4.2. Effects of plant maturity prior to chilling on flowering

Plants of *R. floribunda* were competent to perceive chilling as one-day old seedlings and they did flower. This suggests a short juvenile phase of this species. Cave & Johnston (2010) stated that the short juvenility stage indicates an ephemeral trait, and capacity to promote flowering by exposing plants to chilling can be utilized for commercial production by shortening production time. In other ornamental plant species such as *Cineraria*, plants were not be able to perceive chilling stimulus for floral development until the plants reach 6 - 7 leaves (cv. 'Cindy Blue') or 7 - 8 leaves (cv. 'Cindy Dark Red') (Yeh & Atherton 1997).

Although there was not clear difference for *R. floribunda* with regards to time to first VB and inflorescence number at week 6 among age groups, the number of days to anthesis and inflorescence numbers at 12 and 23 weeks indicated that older plants prior to chilling showed more floral development (Table 3.1). In addition, inflorescence dry weight was higher for older plants. These are consistent with the study results of Markowski & Ryka (1981) and Townsend (1982) in which the older plants prior to cold induction showed higher floral production. According to Cave & Johnston (2010), the increased floral production in older plant group might be due to the longer periods for branching and development.

5. CONCLUSION

Rhodanthe floribunda has a facultative requirement to low temperature. With regard to chilling duration, the longest chilling period (3 weeks) was found to be the most effective treatment which induced faster development rate and more floral production under 11h day-length. Generally, the oldest plant group (4-weeks old) prior to chilling treatment reached higher values of growth and development parameters.

6. ACKNOWLEDGEMENT

This was part of a project funded by The Australian Flora Foundation and The University of Queensland. Also, we thank Mr. Allan Lisle for his support in the statistical analysis.

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